Winter 4-26-2020

Leptin's Role in the Obesity Epidemic

Alyssa C. Washeleski
Wayne State University, alyssacwash@yahoo.com

Alyssa Washeleski

HON 4998

6 September 2019

Leptin's Role in the Obesity Epidemic

1. Introduction

Obesity is a sensitive topic in the United States, and it is not unclear as to why. As further research shows the nation's obesity rate increasing, there are more cases of chronic diseases associated with weight, such as Type II diabetes and heart disease, to prevent. According to the American Medical Association (AMA), in November 2018, the nation's obesity rate was approaching 40% after being around 34-35% between 2005-2012. "Six states—Iowa, Massachusetts, Ohio, Oklahoma, Rhode Island, and South Carolina—saw statistically significant increases in their adult obesity rates between 2016 and 2017" (Henry, 2018, para. 3).

Additionally, Mississippi and West Virginia were the only states above the 30% mark in ~2008. However, now both of those states, along with Alabama, Arkansas, Iowa, Louisiana, and Oklahoma, have 35% or more of their population battling obesity. Seeing as no state had an adult obesity rate higher than 15% in 1985, and no state was above 20% in 2000, these statistics can be alarming. Adult obesity rates exceed 30% in 29 states and 25% in 48 states, leaving only two states with an obesity rate of less than 25% (Henry, 2018).

It has been an increasingly accepted concept that constant weight loss and weight gain, also known as weight cycling, develops from a failure of willpower or consequent to the modern environment (Friedman, 2014). Many individuals will diet to lose weight,

where at least one-third to two-thirds of them will regain more weight than they have lost within the past four to five years. Gaining additional weight can cause feelings of guilt and failure, which can lead to further restriction and dieting, creating a vicious cycle. Additionally, the availability of fast-food and constant accessibility to calorie-dense, nutrient-poor snack foods are only increasing. Therefore, it is easy to assume that our emotions and environment are the cause of the problem (N.A.). However, while they can play a significant role in our eating patterns, it is not necessarily the sole reason we have so many population groups in the obese category (Friedman, 2014). There have been many studies done on twins, animals, adoption cases, and the clustering of certain traits in a family, that prove obesity is the result of both genetic and environmental factors.

Furthermore, losing weight may not be as simple as some individuals may make it out to be. The results of dieting are often short-lived due to the observation that those who have lost weight often begin to feel hungry with powerful cravings, making it difficult for them not to regain the lost weight, and often, additional weight as well. This begs the question—do our bodies have homeostasis for body weight? If they do, how is it controlled, and are medical professionals able to help patients struggling with obesity overcome it? (Friedman & Halaas, 1998)

2. How leptin came to be discovered

In 1950, researchers Ann M. Ingalls, Margaret M. Dickie, and G. D. Snell at the Jackson Memorial Laboratory in Maine discovered obese young mice in their research stock. Obesity, other than that occurring in yellow mice, is relatively rare for this mammal. Yellow mice can attain weights up to 75-80 grams but usually weigh around 60 grams that then decreases as they age. These mice found in their laboratory stock were different

from the start. The obese members were first recognizable at about 4-6 weeks of age when their bodies were shorter, squarer, and had more massive hindquarters. They then increased in weight so rapidly that by three months old, they weighed almost twice as much as their non-obese siblings. At ten months old, the two obese mice weighed 90 and 75 grams and were still gaining weight. Data obtained from the offspring of the siblings concluded that out of 212 mice, 43 of them were obese. It was noted that this 4.9:1 ratio was similar to the 3:1 ratio expected for a recessive gene. This gene was labeled using the symbol *ob* (Ingalls, Dickie, & Snell, 1950).

In 1966, another mutant strain of obese mice called diabetes (*db)* was discovered in the same laboratory. This mutation occurred in an inbred mouse strain and was characterized by a metabolic disturbance similar to diabetes mellitus. This strain was similar to the *ob/ob* mice, except the mice demonstrated symptoms at an earlier age and had a shortened life span. It was noted that this gene was inherited as an autosomal recessive as well.

Permitted studies of anatomical joining normal mice with mutant mice, known as parabiosis, were completed. This procedure would show if a bodily substance were involved in the regulation of satiety and adiposity (Castracane V.D., 2006). It was found that unions of diabetic (*db*) mice with normal mice resulted in death for the normal mouse within 3-4 weeks after surgery. The regular mouse appeared to have died from starvation, alluding to the idea that the diabetic mouse may produce, but not respond to, a satiety factor that prevents overeating. This would affect the regular mouse by inhibiting its intake and therefore producing starvation as a result. When the obese mice were paired

with diabetic mice, the obese partner lost weight and died of starvation. However, when the obese mice were paired with normal mice, they both survived.

Furthermore, the obese mice paired with the normal mice ate less and gained weight less rapidly than obese mice that were put in pairs. These two studies show that the obese partner may not produce enough satiety factor to control its appetite, but that their satiety centers are responsive to the satiety factor that the regular partner provides. In contrast, the *db* mouse may produce satiety factor, but their satiety centers are not functioning to receive it; this may make the body overwork to produce even more satiety factor that is still not recognized. If the *ob* mouse is not able to produce enough satiety factor, and the *db* mouse produces a satiety factor that it cannot respond to, both types of defective mice would over-eat. This would explain the identical obese-hyperglycemic syndromes produced by the two unrelated genes (Coleman, 1973).

In 1978, Dr. Douglas Coleman found that both the *db/db* and *ob/ob* syndromes included signs such as extreme hunger, some degree of hyperinsulinemia, hyperglycemia (temporary and long-term), and marked obesity. Thermoregulatory defects, functional sterility, and hypogonadism were also typical features. All of this evidence supported a hypothalamic defect, seeing as the hypothalamus regulates body temperature, hunger, and sexual behavior (Coleman, 1978).

Dr. Jeffrey Friedman and his team in 1994 were able to isolate and clone the *ob* gene and identify the satiety signal that others had been referencing. The *ob* gene was found to encode mRNA and be the instruction manual for a secreted protein, documented originally as the Ob protein. They officially named this protein leptin… “derived from

the Greek root *leptos*, meaning thin" (Castracane V.D., 2006, p. 6). Leptin was found to be produced in white adipose tissue (Castracane V.D., 2006).

In 1998, Clément identified her first patients with homozygous mutations in the LEPR gene. These patients had no pubertal development as well as a decreased secretion of growth hormone and insulin-like growth factor-I (IGF-I). Usually, obese children would have a decreased secretion of growth hormone in response to stimulation, but they can have accelerated growth as well as increased IGF-I levels. However, for these homozygous individuals, their concentrations of IGF-I, as well as IGF-binding protein 3 (IGF-BP3), were both decreased. Those who were heterozygous or homozygous for the mutation had a mutation in the leptin receptor mRNA caused by the skipping of exon 16; this caused the lack of both the transmembrane and the intracellular domains of the leptin receptor. The mutation in the leptin receptor mRNA did not affect weight regulation managed by leptin in those with the heterozygous mutation. However, for those who were homozygous, their birth weight was average, but severe obesity set in within the first few months of their lives. They did not go through puberty, but those that were homozygous were able to have healthy reproductive functions (Clement et al., 1998).

3. Leptin: What is it?

According to the Dictionary of Science, leptin is "a protein hormone comprised of 167 amino acids in humans that is secreted by adipose tissue and regulates adipose tissue mass and energy balance" (Daintith & Martin, p. 474). The chief function of leptin (often referred to as the 'satiety hormone' or 'obesity hormone') is to communicate to the brain and other organs that there are sufficient fat stores and that the organism is not starving.

This hormone diminishes the drive to consume nourishment while also allowing the body to expend energy through sympathetic nerve activity (Friedman, 2014; Park & Ahima, 2015). When leptin is not present, or when there are signs of leptin resistance, or when there are reduced levels as commonly seen after fasting, the body will adjust accordingly and reduce energy expenditure as well as stimulate appetite (Friedman, 2014). Additionally, these are the metabolic adaptations that occur when one loses weight, and this can make it very difficult to maintain the weight loss. The lowest amounts of leptin in the body are noted at mid-afternoon and the highest at midnight due to leptin's circadian rhythm. Women tend to have higher amounts of leptin than men, which may be attributed to the role of sex steroids or the fact that subcutaneous fat produces more leptin than visceral fat, and women generally have higher amounts of subcutaneous fat deposits than men (Park & Ahima, 2015).

Leptin and hypothalamic neuropeptide Y are essential to maintaining energy homeostasis in the body (Wasim, Awan, Najam, Khan, & Khan, 2016). The leptin hormone acts on the leptin receptors in the hypothalamus and inhibits the expression of neuropeptide Y, thereby inhibiting its appetite-stimulating effects. Having a sizeable amount of leptin in the body also promotes the synthesis of the appetite suppressant known as the melanocyte-stimulating hormone (Friedman, 2014; Park & Ahima, 2015).

This hormone leptin circulates through our bloodstream and enters our central nervous system (CNS) to interact with its receptor. The leptin receptor is expressed in key brain areas that regulate food intake, energy expenditure, and autonomic function (Meek & Morton, 2012). The receptors leptin binds to are encoded by a specific gene called *LEPR*; there are six isoforms of these receptors, LEPR a-f, resulting from

alternative splicing of the *Lepr* mRNA (Myers et al., 2012; Wasim et al., 2016). These play a crucial role in feeding regulation, with the longest receptor, LEPR-b (or LRb), expressed mainly in the hypothalamus and seen as the primary leptin receptor (Park & Ahima, 2015). The LEPR-b receptor mediates all physiological effects of leptin and is also involved in the signal transduction of leptin (Jung & Kim, 2013; Myers et al., 2012). When leptin binds to LEPR-b, this sets off a series of events, including the phosphorylation and activation of Janus kinase 2 (JAK2), tyrosine residues, and downstream signaling proteins, such as… "the signal transducer and activator of transcription 3 (STAT3), phosphatidylinositol 3-kinase (PI3-kinase), and the mitogen-activated protein kinase (MAPK)" (Jung & Kim, 2013, p. 202). STAT3 is the most critical signal transducer since it plays a significant role in the appetite-reducing effect of leptin. It also regulates the transcriptional activity of multiple genes, including proopiomelanocortin (POMC), neuropeptide Y (NPY), agouti-related peptide (AgRP), and the signaling inhibitor suppressor of cytokine signaling-3 (SOCS3). Increased expression of SOCS3 inhibits leptin signaling because it will bind to the LRb-JAK2 complex; this creates an inhibitory feedback loop for leptin signaling (Jung & Kim, 2013).

Leptin receptors belong to the glycoprotein 130 (gp130) family of cytokines (Wasim et al., 2016). Leptin is also seen as a form of cytokine. A cytokine is essentially a small protein that is secreted from certain cells and can have an effect on cell behavior. (Hine, 2019). Specifically, leptin is seen as an adipokine; this is a hormone with cytokine-like actions that is secreted from adipose tissue. It plays a role in glucose and lipid homeostasis, immunity, inflammation, and bone physiology, but its most potent

effect is regulating body weight and energy balance (Paz-Filho, Mastronardi, & Licinio, 2015). Therefore, any mutation in the LEP and LEPR could have a significant influence on the body's metabolism or homeostasis, potentially leading to obesity (Wasim et al., 2016).

Other than energy regulation, leptin is also involved in numerous other tasks in the body, such as insulin sensitivity, sexual maturity, lipid metabolism, bone metabolism, and immune function. Leptin inhibits the gene expression for insulin and insulin secretion encouraged by glucose, forcing the body to turn to its body fat stores for glucose. Inadequate nutrition can result in delayed sexual maturation; however, in rodent studies, leptin administration was shown to help rodents with decreased food intake still advance into adolescence. Because of this, leptin is observed to be an important indicator that a child is prepared to hit puberty and go through sexual maturation (Gruaz et al., 1998). Central leptin administration also inhibits lipogenesis and instead encourages the breakdown of fat cells in adipose tissue and the liver. Additionally, leptin stimulates fatty acid oxidation, thereby decreasing the amount of fat stored within white adipocytes and the liver. Studies have shown that “leptin acts mainly in the brain to influence glucose and lipid metabolism” (Park & Ahima, 2015, p. 7). As for bone metabolism, in individuals diagnosed with hypothalamic amenorrhea, we see that leptin treatment increases markers for bone formation and bone mineral density. Leptin has been known to encourage osteoblast proliferation and mineralization, as well as boost osteoblast differentiation, a crucial process for bone homeostasis and repair (Park & Ahima, 2015).

Leptin can also have varying effects on our immunity. Leptin increases macrophage phagocytosis, encouraging the body to get rid of unwanted and potentially

harmful substances in the body. It also stimulates the movement of neutrophils and the production of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α. Although leptin encourages the production of T-helper 1 cells, it does discourage the production of regulatory T-cells; this may affect the chances of developing an autoimmune disease. It was found that leptin increases the likelihood of certain strains of mice developing autoimmune encephalomyelitis, an animal model of multiple sclerosis (MS), and may even accelerate the onset of the disease. Meanwhile, *ob* mice were seen as resistant to the disease. Humans with MS were noted to have increased leptin levels in their blood and cerebrospinal fluid and a reduced number of their regulatory T-cells when compared to controls (Park & Ahima, 2015).

4. Leptin Disorders/Dysfunctions

Leptin gene therapy can be beneficial for those whose bodies produce little to no leptin. Congenital leptin deficiency (CLD) is a condition present from birth that causes severe obesity beginning in the first few months of life. This condition is rare; only eight different mutations of the leptin gene in a total of 34 humans have been noted to produce CLD. These individuals are constantly hungry due to their bodies producing insufficient amounts of leptin (Paz-Filho et al., 2015). As a result, they quickly gain weight after birth. Over time, this leads to hyperphagia and obesity (Montague et al., 1997). It was also found that these individuals have hypogonadotropic hypogonadism, which means sterility due to the inadequate production of hormones that help with sexual development (N.A., 2020).

The first report of CLD was in 1997 involving two Pakistani cousins (Wasim et al., 2016). Serum levels of these family members (Ob1 and Ob2) were measured and

found to be at 1.0 and 0.7 ng/ml. The mean serum leptin levels found in 16 healthy prepubertal children were ~8 ng/ml, and in 30 adults was ~23 ng/ml. Serum leptin levels have a solid positive correlation with obesity, so the findings of barely detectable serum leptin levels were perplexing. This analysis showed that a diagnosis of congenital leptin deficiency was very likely. These children had a history of hyperphagia, due to reports of eating noticeably more than their siblings ever since early infancy. Even more interesting is that when the two sets of parents and all siblings were tested, all four parents and one of the four siblings were heterozygous for the frame-shift mutation in the leptin gene. The parents and siblings were not overweight or exhibited any symptoms as Ob1 and Ob2 did (Montague et al., 1997).

Luckily, this rare deficiency does have a treatment of daily injections of recombinant human leptin. Exogenous administration of leptin can help treat LEP mutations (Wasim et al., 2016). The only form of leptin available for human therapy is known as recombinant methionyl human leptin or metreleptin; this is a pharmaceutical form of leptin and is often administered starting at 0.02-0.04 mg/kg once a day. These intravenous doses of leptin can create significant losses in individuals with CLD in terms of body weight, body mass index (BMI), and fat mass. In one study, those with a mean BMI of ~51.2 kg/m^2 had a mean BMI of ~26.9 kg/m^2 after 18 months of treatment. Most of the weight was from fat loss, which naturally came from the decrease in energy intake due to the individuals reporting less hunger, less desire to consume food, and a greater sense of fullness. It should be noted that the leptin did not increase their energy expenditure; instead, it made it possible for these individuals to maintain the same metabolic rate. It was also noted that "leptin therapy increased 24-h fat oxidation to levels

higher than those of healthy controls under a 9- to 20-wk low-calorie diet" (Paz-Filho et al., 2015, p. 149).

One of the most significant effects seen was on food intake with the diminished insatiable hunger. Leptin therapy was shown to reduce energy intake during a test meal by up to 84%. It was also noted that the patient's food preferences started to show. When one is severely hungry, they will consume anything put in front of them. However, within seven days of leptin administration, patients were able to discriminate between foods they liked and disliked. Energy expenditure was not noted to have changed due to the administered leptin hormone. It is reported that this may be due to abnormalities of sympathetic nerve function in leptin-deficient adults, which would lead to defects in the efferent sympathetic limb of thermogenesis (Farooqi & O'Rahilly, 2014).

Individuals can be genetically partially deficient in leptin by only inheriting one functional copy of the leptin gene. Therefore, these individuals would have lower circulating leptin levels than those with two functional copies of the gene. Usually, we see a positive correlation with the amount of serum leptin concentration and BMI. However, it was found that those who were partially deficient in leptin had lower leptin levels accompanied by an increased body fat percentage when compared to controls of the same ethnicity and BMI (Farooqi et al., 2001).

Lipodystrophies are "a group of rare disorders… characterized by variable loss of body fat" (Hussain & Garg, 2016, p. 1). This disorder could affect the entire body, termed generalized lipodystrophy, or only certain areas of the body, defined as partial lipodystrophy, and can be genetic or acquired. Individuals with generalized lipodystrophy have reduced amounts of leptin circulating in their bodies (Hussain & Garg, 2016).

Metreleptin has been confirmed to reverse the metabolic irregularities that people with lipodystrophy have a higher risk of developing, such as insulin resistance, diabetes, high blood levels of triglycerides, and fatty liver disease. Those who were administered metreleptin 1-2 times per day were found to have significant reductions in fasting blood glucose levels, fasting insulin, hemoglobin A1c, serum triglycerides, total cholesterol, and low-density lipoprotein (LDL) cholesterol. Improvements in liver health were also reported with reductions in alanine transaminase and aspartate transaminase (Paz-Filho et al., 2015).

Hypothalamic amenorrhea, a condition where a female's menstrual cycle stops due to an issue with the hypothalamus gland, can also be helped with leptin replacement therapy (N.A., 2017). In this condition, fat mass is decreased, resulting in reduced serum leptin levels, osteoporosis, and anovulation in women. When leptin replacement therapy is administered, increases in bone mineral density are noted along with improvements in the gonadal axis (Paz-Filho et al., 2015).

5. **Genetic Variants**

Just as rare disorders caused by homologous gene mutations that can affect body weight regulation have been found, there may be more common mutations, similar to those found in the in *ob* and *db* mice, occurring in the human population. "A model in which susceptibility to obesity is determined largely by genetic factors, but the environment determines phenotypic expression" has been proposed (Barsh, Farooqi, & O'Rahilly, 2000, p. 645). It could be possible that many humans have a high genetic susceptibility to increased adiposity, but that their environment, whether caloric availability is restricted or increased in their particular population group, could conclude their results. If this were

found to be accurate, medical professionals could focus on recognizing and assisting those who are more susceptible to obesity through genetics, and the idea that obesity is an individual's choice could be further dismissed. Several potential loci, or location of genes on chromosomes, have been identified through genome-wide linkage scans. Candidate genes are "those whose dysfunction might reasonably be expected to result in obesity by virtue of their having… effects on energy intake, energy expenditure or nutrient partitioning" (Barsh et al., 2000, p. 649). These are found by screening the genetic studies done on mice and determining what genes are suspected of playing a role in obesity. Further research is needed in this area, but hopefully, additional testing and research in the future will help to narrow down if there are genes in humans that are causing specific populations to be more at risk for obesity than others (Barsh et al., 2000).

6. Leptin Resistance

Obesity is characterized by an excessive accumulation of fat in adipose tissue. As indicated previously, leptin, a product of the obese (*ob*) gene, is expressed from these adipocytes. Due to this, those who are obese display high levels of leptin circulating in their bloodstream. One would think that these individuals would feel highly satiated, but the mere fact that they remain obese shows that these high leptin levels fail to reduce excess adiposity (Park & Ahima, 2015). Regardless of these higher levels, leptin can fail to retain its appetite-suppressing effects of reducing weight in certain obese subjects if the receptors are not able to accept the hormone or become resistant (Wasim et al., 2016).

Leptin resistance is quite similar to an individual developing type II diabetes. Just as a person with type II diabetes becomes insulin-resistant, those with leptin resistance have a diminished response to the excess secretion of leptin (Jung & Kim, 2013); this has

been defined as ‘leptin resistance,’ although it has been controversial what exactly falls under this term. The presence of high levels of leptin in obesity, as well as the ineffectiveness of exogenous leptin to provide any metabolic assistance in the body of obese individuals, have both been considered to fall under this term (Myers et al., 2012). Obese people are insensitive to large amounts of endogenous leptin as well as exogenous leptin treatment. Throughout evolution, low levels of leptin have been an important indicator to fight against starvation, as that presents a much larger threat to survival than overnutrition does. Consequently, there may be only an absolute maximum concentration of leptin that can do its job properly, making any positive effects from the administration of endogenous leptin impossible (Park & Ahima, 2015). It is also important to note that the leptin resistance caused by the excess adipose tissue will further aggravate the adipose tissue and lead to intense hunger cues, perpetuating a vicious cycle (Jung & Kim, 2013).

The more information discovered about leptin resistance, the clearer it becomes that this condition may be resulting from a multi-factorial issue. Leptin plays a substantial role in the cause of obesity and how it progresses, although the exact mechanisms have not yet been established (Farr, Gavrieli, & Mantzoros, 2015). What we do know is that leptin resistance may point to two main culprits: issues with the leptin receptor not signaling correctly to the rest of the body or impaired transport of leptin to the brain (Jung & Kim, 2013).

Leptin is transported to the brain through two mechanisms: the brain-cerebrospinal fluid (CSF) barrier and the brain-blood barrier (BBB). It then gets received to the leptin receptors (LRs) that are highly concentrated in the hypothalamus. The two

specific brain cell groups that leptin targets are 1) the area secreting neuropeptide Y (NPY) and agouti-related peptide (AgRP) (both being appetite-stimulating peptides) and 2) the area synthesizing proopiomelanocortin (POMC), which is a forerunner of the anorexigenic (reduces appetite) α-melanocyte stimulating hormone (α–MSH). These two clusters of neurons are both located in the arcuate nucleus (ARC). Leptin suppresses the activity of NPY/AgRP neurons which would usually compete with α–MSH while also activating POMC neurons (Paz-Filho et al., 2015)

Impaired Leptin Transport

Leptin transport may be impaired in the following ways. We know that in obese individuals, regardless of their high plasma levels, their ability to transport leptin to the cerebrospinal fluid is decreased in comparison to individuals with less body fat. Since leptin enters the brain via a saturable transport system, the transporters are likely overwhelmed. Additionally, fatty acids and the cytokine, tumor necrosis factor-α, have been shown to impair leptin transport into the brain. With obese individuals having increased amounts of both of these substances, this may be playing an additional role in impaired leptin transport (Paz-Filho et al., 2015).

Impaired Leptin Signaling

Phosphorylated STAT3, one of the critical roles in the leptin cascade effect, has been noted to be decreased in the hypothalamus of diet-induced obese (DIO) mice. Increased amounts of SOCS3 noted in obese mice would have inhibited the effects of leptin, with the result being unsatiated individuals. This idea is further strengthened by mice who had decreased leptin resistance with the deletion of the SOC3 gene. The weakened signaling

arises specifically in the ARC, implying that this may be the area of the hypothalamus that allows leptin resistance to occur (Jung & Kim, 2013). It was also discovered that mice with a deficiency of the protein tyrosine phosphatase 1B (PTP1B) showed improved leptin and insulin sensitivity and were additionally resistant to weight gain when consuming a high-fat diet (Paz-Filho et al., 2015).

Endoplasmic Reticulum Stress

Stress on the endoplasmic reticulum (ER), the primary source of newly created and packaged proteins, could also lead to issues regarding leptin (Paz-Filho et al., 2015). The ER's homeostasis is upheld by "balancing ER loading of nascent proteins with capacity to fold these proteins" (Jung & Kim, 2013, p. 204). When there is an imbalance, this creates what is known as ‘ER stress’ and can activate the unfolded protein response (UPR) (Ozcan et al., 2009). This, if unresolved, can lead to dysfunction and even the death of cells. It can also impair proper leptin signaling (Jung & Kim, 2013). ER stress is believed to be caused by overnutrition, increased cytokine levels, or increased free fatty acids levels.

Phospho-PERK is one of the components that signal the UPR stress response. When mice are fed with a high-fat diet versus a regular diet, they have significantly increased PERK phosphorylation. Further testing showed that the ER stress occurred in the hypothalami of the mice who developed obesity, not the mice who stayed lean while also consuming the high-fat diet. Mice injected with tunicamycin, which induces ER stress in the hypothalamus, were found to have inhibited phosphorylation of LEPR-b. The idea that activated UPR signaling blocked the signaling of the leptin receptor was indicated. As for leptin resistance, ER stress that was induced with tunicamycin also

resulted in notably increased mRNA levels of NPY and AgRP. The activation of STAT3, which plays a vital role in the leptin cascade effect, was also inhibited. Additionally, infusing tunicamycin notably increased the food intake of the mice (Ozcan et al., 2009). The combination of these results suggests leptin resistance.

Ozcan and his team then performed experiments to determine if the improved function of the endoplasmic reticulum would enhance leptin signaling. X-box binding protein 1 (XBP1) is one of the primary genes that regulates ER folding capacity. When mice in the control group were given doses of tunicamycin 0.01 μg/ml and higher, activation of LEPR-b was impeded. However, when a mouse infected with an adenovirus encoded for XBP1s was given doses of tunicamycin, the XBP1s increased the cells resistance to tunicamycin doses up to 0.05 μg/ml. The XBP1 mice had increased activation of LEPR-b compared to the control mice, even when they were given higher doses of tunicamycin (0.1 and 1 μg/ml). Activating transcription factor 6α (ATF6), another regulator of ER capacity, was found to increase the resistance of cells to tunicamycin and did not allow LEPR-b signaling to be inhibited.

Furthermore, XBP1 knockout mice (XNKO) were compared against control mice with both consuming a high-fat diet. The XNKO group were found to gain weight more rapidly than the control group throughout the trial and were also found to weigh significantly more by the fourth week of the trial. The XNKO group additionally had a much more significant increase in leptin levels when compared to the slight increase seen in the control mice, with the XNKO mice also being noted to have a significantly lower amount of lean body mass compared to the control group (Ozcan et al., 2009).

Hypothalamic Inflammation

Overconsumption of nutrients over time can lead to inflammation in areas of the body such as the muscle, liver, and adipose tissue, as well as the central nervous system. Increased expression of proinflammatory cytokines like IL-1, IL-6, and TNF-α was noted in the hypothalamus of obese animals (Jung & Kim, 2013). Excessive intake of calories may cause, specifically, chronic low-grade inflammation; this is defined as "the chronic production… [of] a low-grade state of inflammatory factors" (BIM, 2016, para. 2). Instead of the inflammation helping to resolve the issue, it may never go away. Instead of the disease being resolved, the disease is maintained. Obesity is one of the conditions characterized by low-grade inflammation. This inflammation could destroy brain tissue, especially in the hypothalamus, which could be a contributor to leptin resistance. In rodent studies, a high-fat diet was shown to induce inflammation of the hypothalamus, which in turn altered the development of tissue in that section of the brain. The inflammation resulted in the mice having increased food intake, decreased energy expenditure, and increased insulin resistance. It was also found that an increase in calorie consumption led to hypothalamic astrogliosis, which is a defense mechanism of the CNS to minimize and repair damage after injury (Sharma, 2015), and activation of microglial cells, which are a specific form of macrophage that remove damaged neurons (N.A.). High-calorie consumption also caused a reduction of proopiomelanocortin (POMC) neurons, which are turned on by leptin to decrease appetite and increase energy expenditure (Paz-Filho et al., 2015).

7. How to battle leptin resistance

Exogenous Leptin & Leptin Sensitizers

At one point, it was thought that those who were leptin-resistant could be treated by the administration of exogenous leptin. This was a fascinating concept at the time; however, it is now known that this is not the case (Jung & Kim, 2013). Obese individuals treated with up to 0.3 mg/kg/day of exogenous leptin had variable weight loss. Those who had more massive doses of leptin were found to have a more significant change in their weight. In another study, patients were administered the hormone and advised to modify their lifestyle. No changes were noted in their weight. The results were the same for those who completed a follow-up after their gastric bypass surgery.

Additionally, in those with type 2 diabetes, 20 mg/day of metreleptin did not affect their body weight. For those who are not obese, have type 1 diabetes, or have low levels of leptin, exogenous leptin may be useful. It may be helpful since these individuals have a lower amount of adipose tissue and, therefore, would be more leptin sensitive. This drug may become an option in the future for those currently struggling with obesity, but for now, it is unable to produce improvements in weight (Paz-Filho et al., 2015).

Even though exogenous doses of leptin will not help those with leptin resistance, it may help prevent one's metabolic rate from going down while they are losing weight. Leptin exerts its effect in maintaining weight much more than weight loss; it is always fighting against our attempts to lose body fat mass. When obese men achieved a negative energy balance in just four days by exercising and implementing a caloric restriction, their circulating leptin levels decreased. These individuals lost weight and became leptin-deficient due to weight loss; however, exogenous doses of leptin were able to restore thyroid hormone levels, sympathetic nerve activity, and energy expenditure, as well as increase satiation (Park & Ahima, 2015).

A combination of leptin and leptin sensitizers may also help. Metformin, the long-acting glucagon-like peptide extendin-4, and fibroblast growth factor (FGF)-21 were found to improve leptin sensitivity in rodent subjects when given alongside leptin (Paz-Filho et al., 2015). Administration of leptin with exendin-4 or FGF21 produced restored leptin responsiveness in DIO mice after an initial body weight loss of 30%. Additionally, amylin, which is a satiety agent, paired with leptin, helps to reduce body weight and fat mass in diet-induced obese rodents while avoiding the reduction in energy expenditure that occurs when one loses weight. The combination of both of these treatments results in more weight loss in obese subjects than either treatment on its own (Park & Ahima, 2015). When leptin was administered along with insulin in, a compounded effect was noted on the hypothalamic neurons. This combination promoted browning of the white adipose tissue, which would generate heat and therefore allow more calories to burn, advancing weight loss. Additionally, when leptin is combined with liraglutide, a drug designed for type II diabetics, it further helps reduce "cumulative food intake and body weight through reduced meal frequency in lean rats" (Farr et al., 2015, p. 5).

Dietary Compounds

Celastrol, a compound extracted from the 'thunder god vine,' has been shown to strongly affect changes in gene expression that are associated with returning to endoplasmic reticulum homeostasis. This compound was injected into the peritoneal cavities of diet-induced obese (DIO) mice. After three weeks of injections, it was found that the DIO mice had lost a substantial amount of body fat due to having a drastic reduction in food intake that gradually normalized. These effects were not seen in *ob* or *db* mice, alluding that the drug may help increase leptin sensitivity. However, it was noted that the *ob* mice

which were receiving injections of leptin along with the celastrol had reduced food intake; this further alludes to the idea that celastrol may intensify the effects of leptin. Fifteen hours after the celastrol was administered to the DIO mice, their hypothalami showed increased levels of phosphorylated signal transducer and activator of transcription 3 (STAT3), which plays a significant role in the appetite-reducing effect of leptin and regulates the transcriptional activity of multiple genes as stated previously. Celastrol also reduced the levels of phosphorylated eukaryotic translation initiation factor 2α kinase 3 (PERK) in the mice, which is a sign of ER stress. Improved signs of fatty liver disease, lowered cholesterol levels, and improved glucose levels were also noted in the DIO mice (Bray, 2015).

Certain dietary food compounds have been noted to help reduce hyperleptinemia, enhance leptin transport across the BBB, and enhance leptin signaling. Numerous phenolic compounds found in fruits and vegetables, such as resveratrol, oleuropein, neohesperidin, and myricetin, have been found to reduce hyperleptinemia. Even extracts from polyphenolic-rich compounds, such as brown algae, peach and plum juice, and pecans, have had the same effects. Terpenes, found in items such as thyme, tea, tomatoes, watermelon, papaya, and oranges; PUFAs, found in fish oils, beef, lamb, and dairy products; and soluble fiber, such as pectin, have also been effective at reducing circulating leptin levels. It is still unclear what mechanisms are used to yield these reductions, but it has been speculated that the leptin gene may be repressed. In a study performed with the phenolic compound oleuropein, found in olives, it was noted that “a reduction in the levels of serum leptin [was] correlated with a reduction of the expression of the leptin gene” when mice were supplemented with this compound (Aragones, Ardid-

Ruiz, Ibars, Suarez, & Blade, 2016, p. 1795). It should be made known, however, that most of these studies, excluding the one on conjugated linoleic acid (CLA) falling under the PUFA category, were done on rodents.

When looking at food compounds to enhance transport across the BBB, many studies focus on an increase in the expression of megalin, a common leptin transporter. Vitamin A and D and increased secretion of bile acids due to a lithogenic diet, as well as peroxisome proliferator-activated receptor (PPAR)-α and PPAR-γ agonists, have been noted to be proficient at producing megalin. PPAR agonists include items such as PUFAs, coumarins, and flavonoids. Enhancing the expression of clusterin may also help increase transport of leptin, as clusterin is a leptin-binding protein that has megalin as an endocytic receptor. Additionally, clusterin helps regulate leptin signaling in the cell lines that express LEPR-b, which we know is in the hypothalamus. Fraxin, a phenolic compound, has been noted to increase gene expression of clusterin and, therefore, may be potentially useful for increasing leptin transport (Aragones et al., 2016).

As for leptin signaling, leucine, found in items such as soy and beef, is the only food component found to increase the expression of LEPR-b. The best way to measure leptin signaling activity is by measuring the level of pSTAT3, which is the transcription factor that regulates the appetite suppression action of leptin. Teasaponin, found in tea, and ginsenoside Rb1, found in ginseng, were found to increase the level of pSTAT3 in rodents fed a high-fat diet (HFD); these increased levels of pSTAT3 lead to increases in leptin signaling. Resveratrol, found in the skin of grapes; celastrol; caffeine; and taurine, found in shellfish and turkey dark meat, were also found to increase levels of pSTAT3/STAT3. Pectin found naturally in fruit, and fucoxanthin found in brown algae,

increased pSTAT3 levels and AMP-activated protein kinase (AMPK) activity in fat cells. AMPK activity is essential since the pathways it helps coordinate are involved in leptin's appetite suppressing effect. Additionally, compounds such as apigenin, ginsenoside Rb1, teasaponin, taurine, leucine, and yerba mate extracts have been shown to heighten levels of POMC neurons, thereby reducing food intake and body weight (Aragones et al., 2016).

Physical Activity

Exercise also acts as a leptin sensitizer and could be used to increase leptin signaling in human skeletal muscle (Park & Ahima, 2015). When one exercises, there is an acute decrease in leptin. This drop in leptin could signal the body to increase energy intake or decrease energy expenditure to maintain homeostasis; this mechanism would explain why obtaining weight loss is unsuccessful for a majority of individuals. However, if consistent exercise regimens could decrease circulating leptin levels and improve leptin resistance, it is possible that a customized exercise regimen could be used to obtain the maximum benefits of exercise on obesity and leptin resistance.

Currently, there is inconsistent evidence showing that exercise regimens decrease circulating leptin levels in the body (Fedewa, Hathaway, Ward-Ritacco, Williams, & Dobbs, 2018). In a study done in 2013, young obese women were noted to have significantly decreased leptin levels after 12 weeks of various walking programs performed five times per week, including low-, high-, and alternate-intensity training (Mezghanni et al., 2014). Another study published in 2009 noted that obese children 6-11 years of age performing 90 minutes of moderate exercise 3 times per week for 12 weeks did lose weight, and therefore, reduce their levels of leptin. These children did have dietary counseling as well; however, it was noted that these children's results were

equally effective as the children that just had dietary counseling alone (Shalitin et al., 2009). In 2018, a meta-analysis was performed "to provide a quantitative estimate of the magnitude of change in leptin levels following participation in exercise interventions lasting more or equal to two weeks" (Fedewa et al., 2018, p. 1). It was found that continuous exercising training for more or equal to two weeks was associated with a decrease in plasma leptin, regardless of sex and age. No differences were noted in the decrease of leptin levels when comparing aerobic, resistance, and alternating exercise training, as well as intensity, duration, or frequency of the exercise regimen. Decreases in leptin are most often associated with a decrease in body fat percentage; however, chronic exercise training seems to have a decrease in leptin levels regardless of changes in body fat percentage. Overall, an exercise regimen combined with a healthy diet plan is noted to be associated with the most substantial reduction in circulating leptin levels. It is still unclear if the reductions in leptin were due to the body's protective mechanisms, improved leptin sensitivity, or potentially both. Regardless, exercise programs could be an essential tool for reducing body fat percentage and circulating leptin levels, thereby improving leptin sensitivity (Fedewa et al., 2018).

8. Conclusion

The scientific field has come a long way in terms of understanding leptin's important role in weight regulation. From the discovery of leptin, understanding what it affects and regulates in the human body, to learn that over time, excessive consumption of nutrition can lead to obesity and leptin resistance. Some genetic defects can cause the body to produce insufficient amounts of leptin; however, the vast majority of those suffering from problems concerning leptin are overweight or obese. Leptin resistance may develop

throughout various mechanisms, such as impaired leptin transport or signaling, as well as stress on the ER and inflammation of the hypothalamus. New techniques for administering leptin, administration of leptin combined with drugs, hormones, or other substances are being discovered and tested. The research being done on this subject is only increasing and looks promising. However, many of these results concerning the administration of different elements along with leptin still need to be tested on humans. Different dietary components can help reduce hyperleptinemia, enhance leptin transport across the BBB, and enhance leptin signaling. Exercise also looks promising. No doubt, there is still much research to be done. We are unaware of the long-term effects of these different treatment options, as well as if they will work for everyone with a body mass index (BMI) in the overweight or obese range. As research surges onward, more understanding of the pathways and mechanisms involved in leptin resistance will be discovered, along with more treatment options that may improve leptin's effectiveness. The main point of research is to help the growing population live the best possible lives they can with the most up-to-date information. Hopefully, the research done in the future will help future generations to live the healthiest and most prosperous lives yet.

References

Aragones, G., Ardid-Ruiz, A., Ibars, M., Suarez, M., & Blade, C. (2016). Modulation of leptin resistance by food compounds. *Mol Nutr Food Res, 60*(8), 1789-1803. doi:10.1002/mnfr.201500964

Barsh, G. S., Farooqi, I. S., & O'Rahilly, S. (2000). Genetics of body-weight regulation. *Nature, 404*(6778), 644-651. doi:10.1038/35007519

BIM. (2016). Low-grade inflamation and the brain. Retrieved from https://bodyinmind.org/low-grade-inflammation-brain/

Bray, N. (2015). Obesity: Reversing resistance to leptin in obesity. *Nat Rev Drug Discov, 14*(7), 458-459. doi:10.1038/nrd4671

Castracane V.D., H. M. C. (2006). The Obese (*ob/ob*) Mouse and the Discovery of Leptin. *Endocrine Updates, 25*. doi:https://doi.org/10.1007/978-0-387-31416-7_1

Clement, K., Vaisse, C., Lahlou, N., Cabrol, S., Pelloux, V., Cassuto, D., . . . Guy-Grand, B. (1998). A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature, 392*(6674), 398-401. doi:10.1038/32911

Coleman, D. L. (1973). Effects of parabiosis of obese with diabetes and normal mice. *Diabetologia, 9*(4), 294-298. doi:10.1007/bf01221857

Coleman, D. L. (1978). Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. *Diabetologia, 14*(3), 141-148. doi:10.1007/bf00429772

Daintith, J., & Martin, E. Dictionary of Science (6th Edition). In (pp. 19): Oxford University Press.

Farooqi, I. S., Keogh, J. M., Kamath, S., Jones, S., Gibson, W. T., Trussell, R., . . . O'Rahilly, S. (2001). Partial leptin deficiency and human adiposity. *Nature, 414*(6859), 34-35. doi:10.1038/35102112

Farooqi, I. S., & O'Rahilly, S. (2014). 20 years of leptin: human disorders of leptin action. *J Endocrinol, 223*(1), T63-70. doi:10.1530/JOE-14-0480

Farr, O. M., Gavrieli, A., & Mantzoros, C. S. (2015). Leptin applications in 2015: what have we learned about leptin and obesity? *Curr Opin Endocrinol Diabetes Obes, 22*(5), 353-359. doi:10.1097/MED.0000000000000184

Fedewa, M. V., Hathaway, E. D., Ward-Ritacco, C. L., Williams, T. D., & Dobbs, W. C. (2018). The Effect of Chronic Exercise Training on Leptin: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Sports Med, 48*(6), 1437-1450. doi:10.1007/s40279-018-0897-1

Friedman, J. M. (2014). 20 YEARS OF LEPTIN: Leptin at 20: an overview. *Journal of Endocrinology, 223*(1), T1-T8. doi:https://doi.org/10.1530/JOE-14-0405

Friedman, J. M., & Halaas, J. L. (1998). Leptin and the regulation of body weight in mammals. *Nature, 395*(6704), 763-770. doi:10.1038/27376

Gruaz, N. M., Lalaoui, M., Pierroz, D. D., Englaro, P., Sizonenko, P. C., Blum, W. F., & Aubert, M. L. (1998). Chronic administration of leptin into the lateral ventricle induces sexual maturation in severely food-restricted female rats. *J Neuroendocrinol, 10*(8), 627-633. doi:10.1046/j.1365-2826.1998.00247.x

Henry, T. A. (2018). Adult obesity rates rise in 6 states, exceed 35% in 7. Retrieved from https://www.ama-assn.org/delivering-care/public-health/adult-obesity-rates-rise-6-states-exceed-35-7

Hine, R. (2019). Dictionary of Biology. 8. Retrieved from https://books.google.com/books?id=Ot-RDwAAQBAJ&pg=PT544&dq=any+of+numerous+small+proteins+released+from+a+variety+of+cell+types+that+affect+cell+behavior&hl=en&newbks=1&newbks_redir=0&sa=X&ved=2ahUKEwi51o3ljc3oAhUCbs0KHStND5IQ6AEwAHoECAYQAg#v=onepage&q=any%20of%20numerous%20small%20proteins%20released%20from%20a%20variety%20of%20cell%20types%20that%20affect%20cell%20behavior&f=false

Hussain, I., & Garg, A. (2016). Lipodystrophy Syndromes. *Endocrinol Metab Clin North Am, 45*(4), 783-797. doi:10.1016/j.ecl.2016.06.012

Ingalls, A. M., Dickie, M. M., & Snell, G. D. (1950). Obese, a new mutation in the house mouse. *J Hered, 41*(12), 317-318. doi:10.1093/oxfordjournals.jhered.a106073

Jung, C. H., & Kim, M. S. (2013). Molecular mechanisms of central leptin resistance in obesity. *Arch Pharm Res, 36*(2), 201-207. doi:10.1007/s12272-013-0020-y

Meek, T. H., & Morton, G. J. (2012). Leptin, diabetes, and the brain. *Indian J Endocrinol Metab, 16*(Suppl 3), S534-542. doi:10.4103/2230-8210.105568

Mezghanni, N., Mnif, M., Chtourou, H., Chaabouni, K., Masmoudi, L., Lassoued, A., . . . Mejdoub, H. (2014). Effect of aerobic training on insulin resistance and C-reactive protein (CRP) levels and subcutaneous abdominal in obese women. *Sport Sciences for Health, 10*(2), 111-118. doi:10.1007/s11332-014-0181-1

Montague, C. T., Farooqi, I. S., Whitehead, J. P., Soos, M. A., Rau, H., Wareham, N. J., . . . O'Rahilly, S. (1997). Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature, 387*(6636), 903-908. doi:10.1038/43185

Myers, M. G., Jr., Heymsfield, S. B., Haft, C., Kahn, B. B., Laughlin, M., Leibel, R. L., . . . Yanovski, J. A. (2012). Challenges and opportunities of defining clinical leptin resistance. *Cell Metab, 15*(2), 150-156. doi:10.1016/j.cmet.2012.01.002

N.A. Disordered Eating & Dieting. Retrieved from https://www.nedc.com.au/eating-disorders/eating-disorders-explained/disordered-eating-and-dieting/

N.A. Microglial cells. Retrieved from https://www.nature.com/subjects/microglial-cells

N.A. (2017). What causes amenorrhea? Retrieved from https://www.nichd.nih.gov/health/topics/amenorrhea/conditioninfo/causes

N.A. (2020, March 17). Congenital leptin deficiency. Retrieved from https://ghr.nlm.nih.gov/condition/congenital-leptin-deficiency#definition

Ozcan, L., Ergin, A. S., Lu, A., Chung, J., Sarkar, S., Nie, D., . . . Ozcan, U. (2009). Endoplasmic reticulum stress plays a central role in development of leptin resistance. *Cell Metab, 9*(1), 35-51. doi:10.1016/j.cmet.2008.12.004

Park, H. K., & Ahima, R. S. (2015). Physiology of leptin: energy homeostasis, neuroendocrine function and metabolism. *Metabolism, 64*(1), 24-34. doi:10.1016/j.metabol.2014.08.004

Paz-Filho, G., Mastronardi, C. A., & Licinio, J. (2015). Leptin treatment: facts and expectations. *Metabolism, 64*(1), 146-156. doi:10.1016/j.metabol.2014.07.014

Shalitin, S., Ashkenazi-Hoffnung, L., Yackobovitch-Gavan, M., Nagelberg, N., Karni, Y., Hershkovitz, E., . . . Phillip, M. (2009). Effects of a twelve-week randomized intervention of exercise and/or diet on weight loss and weight maintenance, and other metabolic parameters in obese preadolescent children. *Horm Res, 72*(5), 287-301. doi:10.1159/000245931

Sharma, K., Zhang, G., Li, S. (2015). Chapter 11 - Astrogliosis and Axonal Regeneration. 181-196. doi:https://doi.org/10.1016/B978-0-12-801732-6.00011-2

Wasim, M., Awan, F. R., Najam, S. S., Khan, A. R., & Khan, H. N. (2016). Role of Leptin Deficiency, Inefficiency, and Leptin Receptors in Obesity. *Biochem Genet, 54*(5), 565-572. doi:10.1007/s10528-016-9751-z